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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07H 15/252, A61K 31/70 — A1

(11) International Publication Number: WO 99/57126

(43) International Publication Date: 11 November 1999 (11.11.99)

(21) International Application Number: PCT/KR99/00220

(22) International Filing Date: 7 May 1999 (07.05.99)

(30) Priority Data:
1998/16348 7 May 1998 (07.05.98) KR

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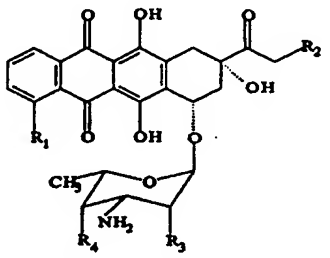
(81) Designated States: BR, CA, CN, HU, ID, IN, JP, KR, LV, MX, RU, TR, US, VN, YU, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: NOVEL ANTHRACYCLINE DERIVATIVES AND THEIR PREPARATION



(I)

(57) Abstract

Disclosed are novel anthracycline derivatives, represented by formula (I), (wherein R_1 and R_4 which may be the same or different, each is a hydrogen atom, methoxy or hydroxy; R_2 is L-aspartate or pyruvate; and R_3 is a hydrogen atom or fluorine atom), which have potent antitumor activity against a broad spectrum of tumors with greatly reduced cardiac toxicity, and a preparation method therefor. The compounds can complement the NAD/NADH ratio balance in vivo to control the activity of oxygen radical-producing enzymes. The compounds can be prepared by linking L-aspartate or pyruvate via an ester bond to the position 14 of conventional anthracycline glycosides. By virtue of the potent activity and pharmaceutical safety, the compounds can be used as pharmaceutically effective ingredients for antitumoral agents.

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NOVEL ANTHRACYCLINE DERIVATIVES AND THEIR PREPARATION

Technical Field

The present invention relates to novel anthracycline derivatives and a method for preparing the anthracycline derivatives. More particularly, the present invention relates to novel anthracycline derivatives which are of potent activity against various cancers with greatly reduced cardiac toxicity and to a preparing method therefor. Also, the present invention is concerned with an antitumor agent comprising the anthracycline derivatives as pharmaceutically effective ingredients.

Prior Art

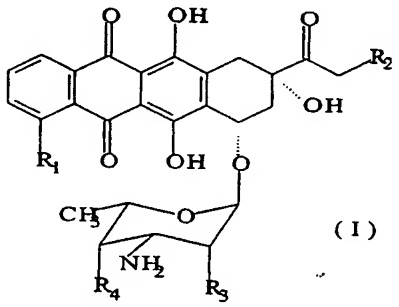
Rhodomycin, daunomycin and adriamycin, which all are of anthracyclines, can be obtained from the fermented broth of Actinomyces species. Since the determination of their chemical structures, much effort has been made to prepare the anthracyclines chemically because they are known to have a broad spectrum antitumor activity. Now, a variety of anthracyclines are developed, which are exemplified by daunomycin, adriamycin, carminomycin, 4′-epi-adriamycin, 4′-methoxyadriamycin, 4′-deoxyadriamycin and idarubicin. Currently, these compounds are clinically used in anticancer chemotherapy.

The quinone compounds with such an anthracycline chemical structure have a potent activity against a wide range of malignant tumors, including lymphocytic leukemia and malignant lymphoma, but are accompanied by serious side effects, such as cardiac problem, bone marrow depression, alopecia, etc. Especially, serious cardiac toxicity frequently appears upon their use. This cardiac toxicity runs an acute course or passes into a chronic state, causing heat contractile dysfunction, arrhythmia and hypotension. What is the worst, the patients treated with these compounds may suffer from heart failure resulting in death. They are, thus, strictly limited in clinical use.

Disclosure of the Invention

Based on the prior invention (Korean Pat. Appl'n Nos. 94-012769, 98-016349) of the present inventors, which discloses that aspartic acid and its salt function to the NADH/NAD ratio in vivo to reduce the cardiac toxicity attributable to doxorubicin, the present invention was developed to suggest novel anthracyclinon-aspartate derivatives, represented by the following structural formula I, which retain the anticancer activity of the quinone derivatives with greatly reduced cardiac toxicity. In addition, the present inventors applied pyruvate, which can control the NADH/NAD ratio in vivo, for anthracyclines to suggest pyruvate-conjugated anthracycline derivatives.

Therefore, it is a primary object of the present invention to provide novel anthracycline glycosides, which have potent anticancer activity and are of even weaker toxic in general, and in particular significantly less cardiac toxicity than the conventional anthracycline glycoside anticancer agents, such as daunomycin, adriamycin, carminomycin and idarubicin, represented by the following structural formula I:



(I)

wherein, R_1 and R_4, which may be the same or different, each is a hydrogen atom, methoxy or hydroxy;

R_2 is L-aspartate or pyruvate; and

R_3 is a hydrogen atom or fluorine atom.

This invention also provides pharmaceutically acceptable salts of the novel anthracycline derivatives.

It is believed that the cardiac toxicity of anthracycline anticancer agents is attributed mainly to the oxygen radicals which are produced in the metabolism of the drugs. To solve the problems the oxygen radicals cause, there were developed new anticancer antibiotics which were a little bit lower in toxicity than pre-existing drugs or there were suggested the

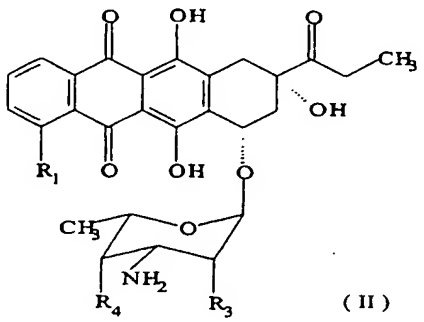
use of antioxidants or radical scavengers (enzymes) in combination with the anticancer agents. U.S. Pat. No. 5,646,177 reports anthracycline derivatives in which glutathione, serving as an antioxidant, is directly linked to the position 7 of the anthracycline. However, these antioxidants and radical-scavenging enzymes are greatly limited in their use because proper conditions are not established for the enzymes included in radical removal and the antioxidants used to prevent radical damage show high reactivity. Accordingly, the demand for novel anthracycline anticancer agents which have potent antitumor activity as well as effective antioxidant activity to reduce the side effects of oxygen radicals in addition to being highly safe, allows the invention to be realized. Also, it is another object of the present invention to provide a process for preparing the novel anthracycline glycoside of the structural formula I or pharmaceutically acceptable salts thereof.

Brief Description of the Drawings

Fig. 1 is a chemical scheme showing the preparation pathway of the novel anthracycline glycoside anticancer compounds according to the present invention.

Best Modes for Carrying Out the Invention

The compounds represented by the structural formula I are prepared by linking L-aspartate or pyruvate via an ester bond to the position 14 of the anthracycline glycoside anticancer antibiotics represented by the following general formula II:



(II)

wherein R_1, R_2, R_3 and R_4 each are as defined above.

The well-known anthracycline glycoside anticancer compounds represented by the general formula II correspond to the starting materials 1a to 1f in the preparation scheme for the novel anthracycline glycoside anticancer compounds of the invention. In accordance with the present invention, the preparation of the novel anthracycline glycoside compounds, represented by the general formula I, is achieved by following the preparation pathway shown in Fig. 1. For the preparation of the novel compounds, well-known techniques (Journal of Medicinal Chemistry, 17, 335, 1974) are applied.

A better understanding of the present invention may be obtained in light of the following examples which are set forth to illustrate, but are not to be construed to limit the present invention. In the following examples, a detailed description will be given for the preparation of adriamycin 14-L-aspartate (3a) and adriamycin 14-pyruvate (4a) from a typical anthracycline compound, daunomycin (1a).

EXAMPLE I

Preparation of 14-Bromodaunomycin hydrochloride (2a)

In a mixture of 50 ml of dry dioxane and 25 ml of methanol was dissolved 1 g of daunomycin hydrochloride which was then added with 10 ml of trimethylorthoformate. After being stirred at room temperature for 20 min, the reaction solution was slowly added with a solution of 0.37 ml of bromine in 3.3 ml of chloroform. The resulting solution was allowed to stand for 40 min and poured at one time in 600 ml of ethyl ether for recrystallization. The precipitates thus obtained were washed with ethyl ether and acetone and dried under vacuum to allow 1.0 g of a red solid: Yield 95%

Melting Point 176-177 °C

IR spectrum (cm^{-1})

3400-3550(hydroxy), 3000-3050(Phenyl), 1690-1720(carbonyl)

^{1}H-NMR spectrum (DMSO-d_6, ppm)

14.04(bs, 1H, Ph-OH), 13.30(bs, 1H, Ph-OH), 7.91(bs, 2H, NH_2), 7.91.(bs, 2H, C_1H, C_3H), 7.89(bs, 1H, C_2H), 5.55(bs, 1H, $C_1{}_{'}H$), 5.28(bs, 1H, $C_{7eq}H$), 4.85(m, 1H, $C_4{}_{'}H$), 4.49(bs, 1H, C_9OH), 4.21(q, J=6.6 Hz, 1H, $C_5{}_{'}H$), 4.00(s, 2H, $C_{14}H$), 3.97(s, 3H, OMe), 3.78(bs, 1H, $C_4{}_{'}OH$), 3.60(bs,

1H, C_3H), 3.05(d, J=18.1Hz, 1H, C_{10eq}H), 2.92(d, J=18.1, Hz, 1H, C_{10ax}H), 2.44(d, J=14.2 Hz, 1H, C_{8eq}H), 2.07(dd, J=14.2, 5.4 Hz, 1H, C_{8ax}H), 1.88(dd, J=12.7, 9.0 Hz, C_2H), 1.85(d, J=12.7, C_2H), 1.65(d, J=6.6, Hz, 3H, C_5Me)

EXAMPLE II

Preparation of Adriamycin-14-L-Aspartate hydrochloride (3a)

In 1 liter of dry acetone were dissolved 1.0 g of 14-bromodaunomycin hydrochloride and 4.0 g of potassium L-aspartate, which were then warmed for 2 hours and cooled to room temperature. The crystals thus obtained were filtered while the solvent was removed under vacuum. The product was re-dissolved in 200 ml of tetrahydrofuran, added with ether hydrochloride and subjected to reaction at -20 °C for 2 hours. The resulting solid was filtered under vacuum and recrystallized in methanol-methylene chloride to allow 0.7 g of adriamycin-14-L-aspartate hydrochloride: Yield 64%

m.p. 189-191 °C

IR spectrum(cm^{-1})

3400-3550(hydroxy), 3000-3050(phenyl), 1725(ester)

^{1}H-NMR spectrum (DMSO-d_6, ppm)

14.01(bs, 1H, Ph-OH), 13.95(bs, 1H, Ph-OH), 8.01(bs, 2H, NH_2), 7.90(dd m, 2H, C_1H, C_3H), 7.64(t, J=8.0 Hz, 1H, C_2H), 5.63(bs, 1H, C_1H), 5.05(bs, 1H, C_{7eq}H), 4.82(m, 1H, C_4H), 4.60(t, J=8.0 Hz, 1H, C_{17}H), 4.05(q, J=6.8 Hz, 1H, C_5H), 4.02(s, 2H, C_{14}H), 3.98(s, 3H, OMe), 3.80(d, J=8.0, Hz, 2H, C_{16}H), 3.68(bs, 1H, C_4OH), 3.28(bs, 1H, C_3H), 2.85(d, J=18.5 Hz, 1H, C_{10eq}H), 2.58(d, J=18.5 Hz, 1H, C_{10ax}H), 2.47(d, J=14.0, Hz, 1H, C_{8eq}H), 2.27(dd, J=14.0, 5.0Hz, 1H, C_{8ax}H)), 2.06(dd, J=12.0, 8.0, Hz, 1H, C_2H), 1.95(d, J=12.0 Hz, 1H, C_2H), 1.26(d, J=6.3, Hz, 3H, C_5Me)

EXAMPLE III

Preparation of Adriamycin-14-Pyruvate hydrochloride (4a)

In 1 liter of dry acetone were dissolved 1.0 g of 14-bromodaunomycin hydrochloride and 3.0 g of sodium pyruvate, which were then warmed for 2 hours and cooled to room temperature. The

crystals thus obtained were filtered while the solvent was removed under vacuum. The product was re-dissolved in 200 ml of tetrahydrofuran, added with etheral hydrochloride and subjected to reaction at -20 °C for 2 hours. The resulting solid was filtered under vacuum and recrystallized in methanol-methylene chloride to allow 0.6 g of adriamycin-14-pyruvate hydrochloride: Yield 58%

m.p. 182-185 °C

IR spectrum(cm^{-1})

3400-3550(hydroxy), 3000-3050(phenyl), 1725(ester)

^{1}H-NMR spectrum (DMSO-d_6, ppm)

13.99(bs, 1H, Ph-OH), 13.29(bs, 1H, Ph-OH), 8.00(bs, 2H, NH_2), 7.90(m, 2H, C_1H, C_3H), 7.69(bs, 1H, C_2H), 5.31(bs, 1H, $C_{1'}$H), 5.30(bs, 1H, C_{7eq}H), 4.81(m, 1H, C_4H), 4.45(s, 1H, C_9OH), 4.32(s, 2H, C_{14}H), 4.10(q, J=6.8, Hz, 1H, $C_{5'}$H), 4.00(s, 3H, OMe), 3.76(bs, 1H, $C_{4'}$H), 3.69(bs, 1H, $C_{3'}$H), 3.12(d, J=18.2 Hz, 1H, C_{10eq}H), 3.07(d, J=18.2, Hz, 1H, C_{10ax}H), 2.51(d, J=14.2, Hz, 1H, C_{8eq}H), 2.27(dd, J=14.2, 5.1Hz, 1H, C_{8ax}H), 2.21(s, 3H, C_{17}H), 1.98(dd, J=13.2, 6.4, Hz, $C_{2'}$H), 1.89(d, J=13.2, Hz, $C_{2'}$H), 1.24(d, J=6.0, Hz, 3H, $C_{5'}$Me)

EXAMPLE IV

Preparation of Carminomycin-14-L-Aspartate hydrochloride (3b)

The same procedures as in Examples I and II were repeated using carminomycin, to give the title compound.

Total Yield 41%

IR spectrum(cm^{-1})

3400-3550(hydroxy), 3000-3050(phenyl), 1730(ester)

^{1}H-NMR spectrum (DMSO-d_6, ppm)

14.01(bs, 1H, Ph-OH), 13.95, 13.89(bs, 2H, Ph-OH), 8.04(bs, 2H, NH_2), 7.92(dd m, 2H, C_1H, C_3H), 7.60(t, J=8.0 Hz, 1H, C_2H), 5.63(bs, 1H, $C_{1'}$H), 4.95(bs, 1H, C_{7eq}H), 4.88(m, 1H, $C_{4'}$H), 4.60(t, J=8.0 Hz, 1H, C_{17}H), 4.02(q, J=6.8 Hz, 1H, $C_{5'}$H), 4.00(s, 2H, C_{14}H), 3.80(d, J=8.0, Hz, 2H, C_{16}H), 3.63(bs, 1H, $C_{4'}$OH), 3.23(bs, 1H, $C_{3'}$H), 2.85(d, J=18.5 Hz, 1H, C_{10eq}H), 2.55(d, J=18.5 Hz, 1H, C_{10ax}H), 2.47(d, J=14.0, Hz, 1H, C_{8eq}H), 2.24(dd, J=14.0, 5.0Hz, 1H, C_{8ax}H)), 2.06(dd, J=12.0, 8.0, Hz, 1H, $C_{2'}$H), 1.95(d, J=12.0 Hz, 1H, $C_{2'}$H), 1.20(d, J=6.3, Hz, 3H, $C_{5'}$Me)

EXAMPLE V
Preparation of Carminomycin-14-Pyruvate hydrochloride (4b)

The same procedures as in Examples I and III were repeated using carminomycin, to give the title compound.

Total Yield 37%

IR spectrum(cm^{-1})

3400-3550(hydroxy), 3000-3050(phenyl), 1725(ester)

^{1}H-NMR spectrum (DMSO-d_6, ppm)

13.93(bs, 1H, Ph-OH), 13.38, 13.29(bs, 2H, Ph-OH), 8.07(bs, 2H, NH_2), 7.93(m, 2H, C_1H, C_3H), 7.69(bs, 1H, C_2H), 5.31(bs, 1H, $C_{1'}H$), 5.30(bs, 1H, $C_{7eq}H$), 4.70(m, 1H, $C_{4'}H$), 4.45(s, 1H, C_9OH), 4.30(s, 2H, $C_{14}H$), 4.10(q, J=6.8, Hz, 1H, $C_{5'}H$), 3.75(bs, 1H, $C_{4'}OH$), 3.69(bs, 1H, $C_{3'}H$), 3.10(d, J=18.2 Hz, 1H, $C_{10eq}H$), 3.05(d, J=18.2, Hz, 1H, $C_{10ax}H$), 2.50(d, J=14.2, Hz, 1H, $C_{8eq}H$), 2.25(dd, J=14.2, 5.1Hz, 1H, $C_{8ax}H$), 2.20(s, 3H, $C_{17}H$), 1.98(dd, J=13.2, 6.4, Hz, $C_{2'}H$), 1.87(d, J=13.2, Hz, $C_{2'}H$), 1.24(d, J=6.0, Hz, 3H, $C_{5'}Me$)

EXAMPLE VI
Preparation of Idarubicin-14-L-Aspartate hydrochloride (3c)

The same procedure as in Example IV was repeated using idarubicin, to give the title compound.

Total Yield 39%

IR spectrum (cm^{-1})

3400-3550(hydroxy), 3000-3050(phenyl), 1730(ester)

^{1}H-NMR spectrum (DMSO-d_6, ppm)

14.00(bs, 1H, Ph-OH), 13.95(bs, 1H, Ph-OH), 8.01(bs, 2H, NH_2), 7.90(m, 3H, C_1H, C_3H, C_4H),), 7.64(t, J=8.0 Hz, 1H, C_2H), 5.60(bs, 1H, $C_{1'}H$), 5.15(bs, 1H, $C_{7eq}H$), 4.90(m, 1H, $C_{4'}H$), 4.67(t, J=8.0 Hz, 1H, $C_{17}H$), 4.10(q, J=6.8 Hz, 1H, $C_{5'}H$), 4.02(s, 2H, $C_{14}H$), 3.80(d, J=8.0, Hz, 2H, $C_{16}H$), 3.65(bs, 1H, $C_{4'}OH$), 3.28(bs, 1H, $C_{3'}H$), 2.80(d, J=18.5 Hz, 1H, $C_{10eq}H$), 2.50(d, J=18.5 Hz, 1H, $C_{10ax}H$), 2.45(d, J=14.0, Hz, 1H, $C_{8eq}H$), 2.25(dd, J=14.0, 5.0Hz, 1H, $C_{8ax}H$)), 2.10(dd, J=12.0, 8.0, Hz, 1H, $C_{2'}H$), 1.90(d, J=12.0 Hz, 1H, $C_{2'}H$), 1.22 (d, J=6.3, Hz, 3H, $C_{5'}Me$)

EXAMPLE VII

Preparation of Idarubicin-14-Pyruvate hydrochloride (4c)

The same procedure as in Example V was repeated using idarubicin, to give the title compound.
Total Yield 42%
IR spectrum(cm^{-1})
3400-3550(hydroxy), 3000-3050(phenyl), 1725(ester)
^{1}H-NMR spectrum (DMSO-d_6, ppm)
13.90(bs, 1H, Ph-OH), 13.39(bs, 1H, Ph-OH), 8.00(bs, 2H, NH_2), 7.93(m, 3H, C_1H, C_3H, C_4H), 7.65(bs, 1H, C_2H), 5.30(bs, 1H, C_1H), 5.30(bs, 1H, $C_{7eq}H$), 4.70(m, 1H, C_4H), 4.45(s, 1H, C_9OH), 4.30(s, 2H, $C_{14}H$), 4.10(q, J=6.8, Hz, 1H, C_5H), 3.76(bs, 1H, C_4OH), 3.65(bs, 1H, C_3H), 3.12(d, J=18.2 Hz, 1H, $C_{10eq}H$), 3.07(d, J=18.2, Hz, 1H, $C_{10ax}H$), 2.51(d, J=14.2, Hz, 1H, $C_{8eq}H$), 2.27(dd, J=14.2, 5.1Hz, 1H, $C_{8ax}H$), 2.21(s, 3H, $C_{17}H$), 1.98(dd, J=13.2, 6.4, Hz, C_2H), 1.89(d, J=13.2, Hz, C_2H), 1.24(d, J=6.0, Hz, 3H, C_5Me)

EXAMPLE VIII

Preparation of 4′-Methoxyadriamycin-14-L-Aspartate hydrochloride (3d)

The same procedure as in Example IV was repeated using 4′-methoxydaunomycin, to give the title compound.
Total Yield 32%
IR spectrum(cm^{-1})
3400-3550(hydroxy), 3000-3050(phenyl), 1725(ester)
^{1}H-NMR spectrum (DMSO-d_6, ppm)
14.09(bs, 1H, Ph-OH), 13.95(bs, 1H, Ph-OH), 8.01(bs, 2H, NH_2), 7.90(dd m, 2H, C_1H, C_3H), 7.65(t, J=8.0 Hz, 1H, C_2H), 5.63(bs, 1H, C_1H), 5.05(bs, 1H, $C_{7eq}H$), 4.96(m, 1H, C_4H), 4.60(t, J=8.0 Hz, 1H, $C_{17}H$), 4.05(q, J=6.8 Hz, 1H, C_5H), 4.02(s, 2H, $C_{14}H$), 3.98(s, 3H, OMe), 3.81(s, 3H, C_4OMe), 3.80(d, J=8.0, Hz, 2H, $C_{16}H$), 3.28(bs, 1H, C_3H), 2.80(d, J=18.5 Hz, 1H, $C_{10eq}H$), 2.55(d, J=18.5 Hz, 1H, $C_{10ax}H$), 2.46(d, J=14.0, Hz, 1H, $C_{8eq}H$), 2.25(dd, J=14.0, 5.0Hz, 1H, $C_{8ax}H$)), 2.04(dd, J=12.0, 8.0, Hz, 1H, C_2H), 1.95(d, J=12.0 Hz, 1H, C_2H), 1.23(d, J=6.3, Hz, 3H, C_5Me)

EXAMPLE IX

Preparation of 4′-Methoxyadriamycin-14-Pyruvate hydrochloride (4d)

The same procedure as in Example V was repeated using 4′-methoxydaunomycin, to give the title compound.

Total Yield 38%

IR spectrum(cm^{-1})

3400-3550(hydroxy), 3000-3050(phenyl), 1725(ester)

^{1}H-NMR spectrum (DMSO-d_6, ppm)

13.95(bs, 1H, Ph-OH), 13.27(bs, 1H, Ph-OH), 8.05(bs, 2H, NH_2), 7.90(m, 2H, C_1H, C_3H), 7.67(bs, 1H, C_2H), 5.31(bs, 1H, C_1H), 5.30(bs, 1H, C_{7eq}H), 4.85(m, 1H, C_4H), 4.45(s, 1H, C_9OH), 4.30(s, 2H, C_{14}H), 4.10(q, J=6.8, Hz, 1H, C_5H), 4.00(s, 3H, OMe), 4.10(s, 3H, C_4OMe), 3.67(bs, 1H, C_3H), 3.12(d, J=18.2 Hz, 1H, C_{10eq}H), 3.07(d, J=18.2, Hz, 1H, C_{10ax}H), 2.50(d, J=14.2, Hz, 1H, C_{8eq}H), 2.24(dd, J=14.2, 5.1Hz, 1H, C_{8ax}H), 2.20(s, 3H, C_{17}H), 1.98(dd, J=13.2, 6.4, Hz, C_2H), 1.86(d, J=13.2, Hz, C_2H), 1.20(d, J=6.0, Hz, 3H, C_5Me)

EXAMPLE X

Preparation of 4′-Deoxyadriamycin-14-L-Aspartate hydrochloride (3e)

The same procedure as in Example IV was repeated using 4′-deoxydaunomycin, to give the title compound.

Total Yield 34%

IR spectrum(cm^{-1})

3400-3550(hydroxy), 3000-3050(phenyl), 1725(ester)

^{1}H-NMR spectrum (DMSO-d_6, ppm)

14.05(bs, 1H, Ph-OH), 13.95(bs, 1H, Ph-OH), 8.01(bs, 2H, NH_2), 7.90(dd m, 2H, C_1H, C_3H), 7.64(t, J=8.0 Hz, 1H, C_2H), 5.63(bs, 1H, C_1H), 5.05(bs, 1H, C_{7eq}H), 4.60(t, J=8.0 Hz, 1H, C_{17}H), 4.05(q, J=6.8 Hz, 1H, C_5H), 4.02(s, 2H, C_{14}H), 3.97(s, 3H, OMe), 3.80(d, J=8.0, Hz, 2H, C_{16}H), 3.26(bs, 1H, C_3H), 2.85(d, J=18.5 Hz, 1H, C_{10eq}H), 2.55(d, J=18.5 Hz, 1H, C_{10ax}H), 2.46(d, J=14.0, Hz, 1H, C_{8eq}H), 2.27(dd, J=14.0, 5.0Hz, 1H, C_{8ax}H)), 2.16(m, 2H, C_2H, C_4H), 2.05(m, 2H, C_2H, C_4H),) 1.29(d, J=6.3, Hz, 3H, C_5Me)

EXAMPLE XI

Preparation of 4′-Deoxyadriamycin-14-Pyruvate hydrochloride (4e)

The same procedure as in Example V was repeated using 4′-deoxydaunomycin, to give the title compound.
Total Yield 38%
IR spectrum(cm^{-1})
3400-3550(hydroxy), 3000-3050(phenyl), 1725(ester)
^{1}H-NMR spectrum (DMSO-d_6, ppm)
13.97(bs, 1H, Ph-OH), 13.32(bs, 1H, Ph-OH), 8.00(bs, 2H, NH_2), 7.90(m, 2H, C_1H, C_3H), 7.65(bs, 1H, C_2H), 5.30(bs, 1H, $C_{1'}$H), 5.30(bs, 1H, C_{7eq}H), 4.45(s, 1H, C_9OH), 4.30(s, 2H, C_{14}H), 4.10(q, J=6.8, Hz, 1H, $C_{5'}$H), 3.75(bs, 1H, $C_{4'}$H), 3.69(bs, 1H, $C_{3'}$H), 3.18(d, J=18.2 Hz, 1H, C_{10eq}H), 3.06(d, J=18.2, Hz, 1H, C_{10ax}H), 2.55(d, J=14.2, Hz, 1H, C_{8eq}H), 2.27(dd, J=14.2, 5.1Hz, 1H, C_{8ax}H), 2.20(s, 3H, C_{17}H), 2.07(m, $C_{2'}$H, $C_{4'}$H), 1.97(m, $C_{2'}$H, $C_{4'}$H), 1.24(d, J=6.0, Hz, 3H, $C_{5'}$Me)

EXAMPLE XII

Preparation of 3′-Fluoroadriamycin-14-L-Aspartate hydrochloride (3f)

The same procedure as in Example IV was repeated using 3′-fluorodaunomycin, to give the title compound.
Total Yield 28%
IR spectrum(cm^{-1})
3400-3550(hydroxy), 3000-3050(phenyl), 1725(ester)
^{1}H-NMR spectrum (DMSO-d_6, ppm)
14.00(bs, 1H, Ph-OH), 13.95(bs, 1H, Ph-OH), 7.90(dd m, 2H, C_1H, C_3H), 7.60(t, J=8.0 Hz, 1H, C_2H), 5.63(bs, 1H, $C_{1'}$H), 5.05(bs, 1H, C_{7eq}H), 4.95(m, 1H, $C_{4'}$H), 4.60(t, J=8.0 Hz, 1H, C_{17}H), 4.05(q, J=6.8 Hz, 1H, $C_{5'}$H), 4.00(s, 2H, C_{14}H), 3.90(s, 3H, OMe), 3.80(d, J=8.0, Hz, 2H, C_{16}H), 3.67(bs, 1H, $C_{4'}$OH), 3.58(bs, 1H, $C_{3'}$H), 2.80(d, J=18.5 Hz, 1H, C_{10eq}H), 2.58(d, J=18.5 Hz, 1H, C_{10ax}H), 2.45(d, J=14.0, Hz, 1H, C_{8eq}H), 2.25(dd, J=14.0, 5.0Hz, 1H, C_{8ax}H)), 2.00(dd, J=12.0, 8.0, Hz, 1H, $C_{2'}$H), 1.95(d, J=12.0 Hz, 1H, $C_{2'}$H), 1.25(d, J=6.3, Hz, 3H, $C_{5'}$Me)

EXAMPLE XIII

Preparation of 3′-Fluoroadriamycin-14-L-Pyruvate hydrochloride (4f)

The same procedure as in Example V was repeated using 3′-fluorodaunomycin, to give the title compound.
Total Yield 32%
IR spectrum(cm^{-1})
3400-3550(hydroxy), 3000-3050(phenyl), 1725(ester)
^{1}H-NMR spectrum (DMSO-d_6, ppm)
13.89(bs, 1H, Ph-OH), 13.25(bs, 1H, Ph-OH), 7.90(m, 2H, C_1H, C_3H), 7.65(bs, 1H, C_2H), 5.30(bs, 1H, $C_{1'}$H), 5.230bs, 1H, C_{7eq}H), 4.80(m, 1H, C_4H), 4.45(s, 1H, C_9OH), 4.32(s, 2H, C_{14}H), 4.15(q, J=6.8, Hz, 1H, $C_{5'}$H), 4.05(s, 3H, OMe), 3.78(bs, 1H, $C_{4'}$H), 3.70(bs, 1H, $C_{3'}$H) , 3.12(d, J=18.2 Hz, 1H, C_{10eq}H), 3.05(d, J=18.2, Hz, 1H, C_{10ax}H), 2.50(d, J=14.2, Hz, 1H, C_{8eq}H), 2.25(dd, J=14.2, 5.1Hz, 1H, C_{8ax}H), 2.20(s, 3H, C17H), 1.98(dd, J=13.2, 6.4, Hz, $C_{2'}$H), 1.92(d, J=13.2, Hz, $C_{2'}$H), 1.28(d, J=6.0, Hz, 3H, $C_{5'}$Me)

As a consequence of intensive and thorough tests, the present inventors found that the compounds represented by the general formula I have similar activity against cancers to those of conventional anthracycline glycoside anticancer agents as well as show such a reduced cardiac toxicity as expected. Therefore, the compounds of the general formula I are considered as anticancer agents by virtue of their low cardiac toxicity and superb anticancer activity.

Biological Activity

Similar in anticancer activity as they are to conventional anthracycline anticancer agents, daunomycin and adriamycin, the compounds I of the present invention show greatly reduced cardiac toxicity as confirmed by tests.

TEST EXAMPLE I

Antitumor Effect on Tumor-Bearing Experimental animals

1) Experimental animals

5 week-aged BDF1 mice, purchased from Charles River Company, were implanted subcutaneously at their sides with B16 melanoma tissues. Separately, Lewis lung carcinoma, a solid cancer, was transplanted into the hypodermis from the groin to the armpit of 5 week-aged BDF1 mice.

2) Formulation and Administration of Drugs

Test samples and a control, adriamycin, were dissolved in water for injection and the drugs thus prepared were administered intravenously 4 times in total to the two experimental animal groups, the B16 melanoma mice and the Lewis lung cancer mice, at various doses at intervals of a week at 1, 8, 15 and 22 days after the tumor implantation.

For a control group, physiological saline was used at a dose of 10 ml per kg of weight.

3) Weighing Body Weight

Each of the test mice was examined for its body weight at the day of test group separation and at the day of drug administration. The data for the change of body weight was based on the body weights measured at one day and eight days after experiment.

4) Tumor Growth Inhibition

The tumor volumes of the experimental animals alive were measured at 16 days after cancer transplantation for Lewis lung carcinoma and at 18 days after cancer transplantation for B16 melanoma. The tumor volume was calculated as $1/2ab^2$ where a is the length (mm) of the longer axis and b is the shorter axis of the solid tumor mass measured by a digital caliper.

Tumor growth inhibition (TGI) was calculated according to the following formula:

$$TGI\ (\%) = (1-Vt/Vc) \times 100$$

wherein Vt represents an average tumor volume of a test group administered with drugs and Vc represents an average tumor volume of a control group administered with the solvent.

5) Observation of Viability and Increase in Life Span (ILS)

To examine whether the experimental animals were alive or dead, observation was done two times every day and the viable period of time from the cancer cell introduction to death was represented by days. The observation was continued to a period of 60 days and the animals which were still alive after then were regarded as survivals.

The anticancer efficacy of each of the drugs was evaluated by the ILS, which was obtained by the ratio (%) of the mean survival time in drug-treated animals to that in the control animals. ILS estimated on day 61 after tumor implantation.

6) Test Results for Antitumor Activity *in vivo*

(1) B16 Melanoma

The antitumor activity of the tested drugs against the B16 mouse melanoma transplanted into the hypodermis of BDF1 mice is given in Table 1, below.

As for TGI, all of the novel anthracycline derivatives showed efficacious effects at a dose of 25 or higher mg/kg while adriamycin was effective at a dose of 10 mg/kg only, on the basis of the measurements at 18 days after the cancer inoculation. A maximal TGI was 58% for the mouse group administered with adriamycin at a dose of 10 mg/kg. On the other hand, the novel anthracycline derivatives showed a maximal TGI of 89% at a dose of 50 mg/kg. Therefore, the novel anthracycline derivatives all were found to be superior to adriamycin in inhibiting the growth of B16 melanoma.

Regarding ILS, the novel anthracycline derivatives all were effective for the groups administered at a dose of 25 mg/kg or higher and adriamycin was effective for the groups administered at a dose of 5.0 and 10.0 mg/kg, based on an ILS of 30% or higher. A maximal ILS was found at a dose of 50.0 mg/kg for the novel anthracycline derivatives and 10.0 mg/kg for adriamycin, amounting to 290-585 % and 119 %, respectively.

TABLE 1

Antitumor Effect against B16 Melanoma-Bearing Mice

Sample Cpd.	Dose (mk/kg)	ILS (%)	Nos. of Survival (>60days)	TGI (%)	Weight Changed (g)
Physiological Saline		0			+0.8
Adriamycin	5.0	36	0	15	+0.8
	10.0	119	0	58	+1.2
	20.0	-27	0	62	-2.5
Adriamycin-14-Asp	25	87	2/6	35	+0.7
	50	321	4/6	64	+1.1
	100	277	3/6	83	-0.2

Adriamycin-14-Pyruv.	25 50 100	124 350 275	2/6 4/6 3/6	32 72 80	+0.4 +1.0 -0.5
Carminomycin-14-Asp	25 50 100	131 410 310	2/6 4/6 4/6	35 80 89	+1.2 +1.9 -0.7
Carminomycin-14-Pyruv.	25 50 100	150 425 295	2/6 5/6 4/6	35 85 88	+2.0 +1.5 -0.9
Idarubicin-14-Asp	25 50 100	281 518 350	3/6 5/6 4/6	45 82 88	+2.1 +2.0 +0.8
Idarubicin-14-Pyruv.	25 50 100	250 510 320	3/6 5/6 4/6	46 79 82	+2.5 +1.9 +0.9
4'-Methoxyadriamycin-14-Asp	25 50 100	150 320 210	1/6 3/6 2/6	35 75 80	+1.4 +1.9 -0.9
4'-Methoxyadriamycin-14-Pyruv.	25 50 100	145 290 200	1/6 4/6 3/6	41 82 85	+1.5 +1.6 -0.4
4'-Deoxyadriamycin-14-Asp	25 50 100	145 390 280	2/6 4/6 2/6	56 70 75	+1.1 +1.1 -0.0
4'-Deoxyadriamycin-14-Pyruv.	25 50 100	135 380 275	3/6 4/6 3/6	55 72 79	+1.2 +1.0 -0.5
3'-Fluoroadriamycin-14-Asp	25 50 100	275 535 410	3/6 5/6 3/6	70 89 95	+1.4 +1.2 +0.7
3'-Fluoroadriamycin-14-Pyruv.	25 50 100	260 585 450	3/6 5/6 3/6	75 87 99	+1.0 +1.1 +0.5

(2) Lewis Lung Carcinoma

The anticancer activity of the novel anthracycline derivatives and adriamycin against the Lewis lung carcinoma introduced to the hypodermis of DBF1 mice is given in Table 2, below.

As for TGI, all of the novel anthracycline derivatives showed medicinally efficacious effects at a dose of 25 mg/kg or higher while adriamycin was effective at a dose of 10 mg/kg only, on the basis of measurements at 16 days after the cancer introduction. A maximal TGI was 58% for the mouse group administered with adriamycin at a dose of 10 mg/kg. On the other hand, a tumor-free state was realized in the mice which was administered with the novel anthracycline at a dose of 50 mg/kg.

Regarding ILS, the novel anthracycline derivatives all were effective for the groups administered at a dose of 25 mg/kg or higher, based on an ILS of 30% or higher. A maximal ILS was found at a dose of 50.0 mg/kg for the novel anthracycline derivatives and 10.0 mg/kg for adriamycin, amounting to 245 % and 58 %, respectively.

Taken together, the data obtained above demonstrate that the novel anthracycline derivatives have medicinally effective activity at a broader dose range with a greatly reduced toxicity and are still better in maximal anticancer effects, such as maximal TGI and maximal ILS, than adriamycin.

TABLE 2
Antitumor Effect against Lewis Lung Carcinoma-Bearing mice

Sample Cpd.	Dose (mk/kg)	ILS (%)	Nos. of Survival (>60days)	TGI (%)	Weight Changed (g)
Physiological Saline		0			+0.8
Adriamycin	5	22	0	25	+1.2
	10	58	0	62	+1.0
	20	-29	0	90	-2.0
Adriamycin-14-Asp	25	35	1/6	49	+0.9
	50	81	3/6	72	+1.5
	100	80	3/6	98	+0.4
Adriamycin-14-Pyruv.	25	40	1/6	39	+0.8
	50	85	3/6	75	+1.2
	100	80	3/6	82	+0.1

Carminomycin-14-Asp	25 50 100	62 121 90	2/6 4/6 3/6	39 62 81	+1.2 +1.4 +1.0
Carminomycin-14-Pyruv.	25 50 100	60 132 104	2/6 4/6 3/6	59 79 85	+2.1 +1.5 +0.9
Idarubicin-14-Asp	25 50 100	71 175 155	2/6 4/6 3/6	60 89 97	+1.5 +1.2 +0.8
Idarubicin-14-Pyruv.	25 50 100	74 165 150	2/6 4/6 3/6	71 88 95	+1.5 +1.8 +0.8
4'-Methoxyadriamycin-14-Asp	25 50 100	51 80 45	2/6 3/6 2/6	28 74 89	+1.5 +1.8 +0.9
4'-Methoxyadriamycin-14-Pyruv.	25 50 100	45 85 55	1/6 3/6 2/6	35 81 89	+2.6 +1.0 +0.4
4'-Deoxyadriamycin-14-Asp	25 50 100	40 95 75	1/6 3/6 2/6	21 79 95	+1.8 +1.6 +0.0
4'-Deoxyadriamycin-14-Pyruv.	25 50 100	45 105 78	1/6 3/6 2/6	22 81 94	+1.8 +1.9 +0.5
3'-Fluoroadriamycin-14-Asp	25 50 100	95 232 190	3/6 5/6 4/6	79 89 95	+1.9 +1.6 +0.5
3'-Fluoroadriamycin-14-Pyruv.	25 50 100	90 245 210	4/6 5/6 4/6	55 72 99	+2.1 +1.9 +0.5

As expected from the data of Tables 1 and 2, the novel anthracycline compounds of the present invention show potent activity against cancers, which would be extremely useful as antitumoral agents applicable for clinical practice, alone and in combination with other

conventional anticancer agents.

It is believed that the anthracycline compounds exhibit similar efficacy to those of parent anticancer agents because, when the novel anthracycline represented by the general formula I exert anticancer activity in vivo, they are decomposed at their ester linkage by esterase, remaining as the same forms as the parent compounds. For example, the compound No. 1 of the present invention is decomposed into adriamycin and L-aspartate by an esterase, in vivo, the former exerting anticancer effects.

The novel anticancer drugs were tested for cardiac toxicity as follows.

TEST EXAMPLE II
Assay for Cardiac Toxicity

1) Experimental Animals

Male Sprague-Dawley rats with a weight of about 200 g were used. To protect them from being stressed by environmental conditions and infection, the animal breeding room was maintained at 22 °C under an SPF condition. After being anesthetized with ether, the rats were intraperitoneally injected with the samples once per week for 5 weeks.

As controls, adriamycin and daunomycin each were administered at a dose of 4 mg/kg while the aspartate derivatives and pyruvate derivatives of the anthracycline were administered at a dose of 5 mg/kg and 3.5 mg/kg, respectively, for cardiac toxicity tests.

2) Electrocardiogram Test

The rats were anesthetized with ether, after which the limbs were fixed on a fixing plate. Two electrodes of an electrocardiometer were respectively connected to a right upper site and a left lower site of the chest to record a standard Limb Lead II electrocardiogram. The record was conducted with Grass polygraph (Model 79E, Wide-band AC, preamplifier Model 7P5B) in which cardiac rates, PQ intervals, QRA complexes, ST intervals, QT intervals, TP intervals and T waves were measured.

TABLE 3

ECG and Weight Change by Adriamycin and Novel Compounds

Sample Cpd.	Dose (mg/kg)	Time (weeks)	QT (msec)	ST (msec)	TP (msec)	TW wave (msec)	Body Weight(g)
Physiological Saline		0	25±0.4	12±0.6	70±1.2	234±10.4	122±16.4
		2	24±0.7	14±0.3	70±0.9	235±15.3	195±12.2
		4	23±0.5	12±0.6	72±0.9	239±10.3	249±15.8
		6	25±0.3	11±0.4	69±1.5	235±13.0	261±14.5
Adriamycin	4mg/kg	0	25±0.5	12±0.5	72±1.2	235±10.5	123±13.5
		2	24±0.4	13±0.4	70±0.8	234±12.4	175±20.4
		4	31±0.5	18±0.5	65±0.9	200±12.5	181±7.3
		6	38±0.4	29±0.6	55±1.4	102±11.4	180±16.4
Adriamycin -14-Asp	5mg	0	23±0.6	12±0.4	68±1.9	230±12.5	120±16.4
		2	24±0.3	14±0.4	70±1.5	235±9.4	195±19.2
		4	22±0.4	12±0.5	72±0.8	239±18.4	249±20.9
		6	25±0.4	11±0.1	69±1.2	234±12.2	261±19.7
	25mg	0	2 2±0.5	12±0.4	69±1.4	233±11.3	122±9.4
		2	24±0.7	14±0.2	70±2.4	230±12.5	185±11.6
		4	28±0.4	17±0.3	66±1.4	210±13.5	230±17.3
		6	32±0.8	20±0.7	61±2.1	159±15.0	225±15.7
Adriamycin -14-Pyruv.	4.5mg	0	22±0.3	12±0.4	70±1.8	233±12.6	120±9.4
		2	24±0.4	14±0.6	69±1.9	235±15.6	190±19.7
		4	23±0.5	12±0.3	72±1.2	239±10.5	240±10.4
		6	24±0.8	11±0.6	69±2.1	235±11.9	265±12.3
	22.5mg	0	24±0.5	12±0.4	70±2.5	232±10.5	126±11.3
		2	24±0.6	14±0.6	70±2.7	230±12.7	185±14.4
		4	29±0.3	18±0.3	67±2.9	215±12.4	220±15.1
		6	32±0.4	21±0.4	61±2.1	160±21.1	210±17.1
Carminomycin -14-Asp	5mg	0	24±0.5	12±0.2	70±1.3	230±12.4	124±8.3
		2	24±0.6	14±0.4	69±1.6	234±13.7	185±9.4
		4	23±0.8	12±0.5	71±1.2	239±21.4	230±15.3
		6	23±0.4	11±0.7	69±0.9	235±22.1	259±10.8
	25mg	0	23±0.5	12±0.6	70±2.1	235±12.5	120±16.8
		2	24±0.6	14±0.4	70±2.2	230±11.9	185±13.9
		4	27±0.3	18±0.5	68±0.9	220±23.1	210±10.4
		6	30±0.4	20±0.3	63±1.5	188±22.3	210±11.7
Carminomycin -14-Pyruv.	4.5mg	0	22±0.7	12±0.4	69±1.8	234±10.3	120±13.4
		2	24±0.6	14±0.2	69±2.1	235±10.3	195±12.6
		4	23±0.8	12±0.4	72±2.9	230±21.5	251±13.4
		6	24±0.4	11±0.3	69±3.1	234±10.4	260±15.9

	22.5mg	0	25±0.6	12±0.4	70±2.7	234±12.5	122±13.3
		2	25±0.6	14±0.2	70±3.1	230±10.3	190±16.2
		4	26±0.4	17±0.2	68±1.9	210±10.6	220±15.6
		6	29±0.8	19±0.2	64±2.5	179±12.2	220±15.4
Idarubicin -14-Asp	5mg	0	25±0.6	12±0.4	70±0.9	230±12.3	121±16.7
		2	24±0.7	14±0.3	69±0.8	234±11.0	190±16.4
		4	25±0.4	12±0.3	70±2.2	239±21.1	240±12.3
		6	23±0.5	11±0.4	69±1.5	235±17.0	255±13.1
	25mg	0	25±0.6	12±0.4	70±2.4	230±21.2	120±16.0
		2	26±0.9	14±0.4	70±2.1	235±15.8	190±16.0
		4	27±0.4	17±0.3	65±1.1	210±17.3	220±11.6
		6	29±0.7	19±0.2	64±1.9	170±14.6	215±3.7
Idarubicin -14-Pyruv.	4.5mg	0	25±0.6	12±0.4	70±0.9	234±10.3	122±18.4
		2	24±0.4	14±0.3	70±1.5	230±12.6	195±8.5
		4	23±0.6	12±0.2	69±2.1	235±10.8	250±14.4
		6	24±0.5	11±0.2	70±2.9	235±9.8	260±16.6
	22.5mg	0	24±0.5	12±0.4	70±1.9	234±8.4	122±9.0
		2	24±0.7	14±0.4	70±2.5	235±11.5	185±14.7
		4	28±0.4	16±0.3	67±3.0	220±13.6	220±13.4
		6	30±0.7	18±0.5	64±3.4	175±12.5	215±11.4
4'-Methoxy adriamycin -14-Asp	5mg	0	25±0.6	12±0.4	69±1.7	234±14.5	122±10.0
		2	24±0.6	14±0.3	70±1.8	235±12.4	195±10.9
		4	23±0.3	12±0.3	71±2.5	230±12.5	240±10.5
		6	35±0.9	11±0.6	69±3.2	235±10.1	255±12.4
	25mg	0	24±0.4	12±0.4	70±3.2	234±15.5	122±12.4
		2	24±0.9	14±0.5	70±2.2	235±16.7	185±13.2
		4	27±0.7	18±0.3	66±2.0	200±12.5	195±14.7
		6	31±0.4	21±0.5	62±1.5	161±9.7	190±14.9
4'-Methoxy adriamycin -14-Pyruv.	4.5mg	0	25±0.7	12±0.4	70±0.9	234±7.5	120±16.7
		2	24±0.6	14±0.6	70±1.2	232±9.2	190±16.8
		4	23±0.8	12±0.5	69±1.5	239±10.4	245±14.4
		6	24±0.9	11±0.3	69±0.8	235±10.5	255±16.5
	22.5mg	0	25±0.5	12±0.4	70±0.8	230±9.5	122±14.3
		2	26±0.6	14±0.1	69±1.5	235±12.5	185±15.5
		4	27±0.4	17±0.2	65±1.8	195±8.5	195±14.4
		6	31±0.4	20±0.3	60±2.3	160±9.9	195±17.6
4'-Deoxy adriamycin -14-Asp	5mg	0	25±0.7	12±0.4	69±0.8	234±11.4	122±12.3
		2	24±0.6	14±0.2	70±1.6	235±12.8	195±13.4
		4	23±0.6	12±0.3	69±2.1	235±9.5	249±15.6
		6	35±0.4	11±0.4	69±3.2	230±15.5	261±13.5

	25mg	0	25±0.5	12±0.1	70±2.2	234±0.9	122±13.4
		2	24±0.3	14±0.3	70±2.6	235±12.6	195±16.8
		4	28±0.4	17±0.3	64±0.9	210±11.7	225±15.9
		6	32±0.7	21±0.4	60±0.8	158±0.9	200±19.9
4'-Deoxy adriamycin -14-Pyruv.	4.5mg	0	24±0.8	12±0.3	70±1.5	234±11.5	122±17.7
		2	24±0.9	14±0.4	70±2.5	235±11.7	195±19.5
		4	26±0.6	12±0.5	69±2.1	239±11.9	249±16.4
		6	31±0.4	11±05	69±0.9	235±12.1	261±17.6
	22.5mg	0	25±0.5	12±0.3	70±0.8	234±10.9	122±6.5
		2	24±0.7	14±0.4	70±2.1	235±12.1	195±3.4
		4	27±0.4	17±0.2	63±0.9	200±9.1	210±4.3
		6	32±0.7	20±0.1	59±3.0	155±12.0	205±4.2
3'-Fluoro adriamycin -14-Asp	5mg	0	23±0.4	12±0.3	70±1.2	234±0.9	122±15.5
		2	24±0.6	14±0.4	68±1.7	235±12.3	195±13.8
		4	23±0.8	12±0.3	69±1.9	230±9.6	249±15.8
		6	24±0.4	11±0.2	69±2.2	234±12.0	261±15.9
	25mg	0	25±0.6	12±0.4	70±2.2	234±14.6	122±16.5
		2	24±0.4	14±0.3	70±1.8	235±14.0	195±17.7
		4	27±0.4	15±0.1	68±0.8	220±11.6	215±9.6
		6	39±0.5	17±0.2	64±1.1	175±12.2	210±19.4
3'-Fluoro adriamycin -14-Pyruv.	4.5mg	0	25±0.6	12±0.1	70±1.1	234±12.5	122±17.0
		2	24±0.9	14±0.3	70±1.4	230±11.5	195±17.8
		4	23±0.6	12±0.2	72±1.2	235±10.4	249±16.3
		6	23±0.8	11±0.5	71±2.0	235±8.9	261±19.0
	22.5mg	0	25±0.4	12±0.3	70±0.8	234±11.5	122±16.7
		2	24±0.7	14±0.2	70±1.2	235±12.3	195±12.4
		4	27±0.7	16±0.3	67±1.3	225±8.1	220±10.5
		6	29±0.4	18±0.4	64±2.5	180±11.9	210±10.2

3) Test Results

(1) Weight Changes by Adriamycin and Novel Anthracycline Derivatives

While the control rats, which were treated with no drugs, gradually gained in weight for 6 weeks, this weight gain rate was significantly lowered in the groups administered with adriamycin. This adriamycin-administered groups suffered from serious toxicity symptoms from 2 weeks after administration until to death at 4 weeks after administration.

In contrast, the same weight gain rate as in the groups was observed in the test groups which were administered with the novel anthracycline derivatives. When the dosage was increased 5 times, however, the rats gained in weight to a little less control, as shown in

Table 3. Particularly, the rats which were administered with the novel derivatives at the same equivalent as in adriamycin, behaved like normal rats with no abnormal symptoms over the whole body.

(2) Change in ECG by Adriamycin and the Novel Anthracycline Derivatives

No changes were observed in the ECG of the control group while a typical ECG change was detected in the adriamycin-administered group. From 3 to 4 weeks after administration, the ECG change began, and was aggravated in a recovery stage. At 3 weeks after administration, a serious ECG change was caused and, since then, no ECG changes were observed because the rats of the adriamycin-administered groups died of heart trouble.

The ECG parameters, QT, ST, TP intervals and T wave heights, which underwent extreme changes upon adriamycin administration, were traced in the anthracycline derivative-administered groups. Consequently, the ECG patterns of the anthracycline derivative-administered groups were almost identical to those of normal rats, demonstrating that the novel anthracycline derivatives of the present invention are of very weak cardiac toxicity.

The compounds of the present invention may be administered parenterally or orally in admixture with pharmaceutically acceptable carriers or diluent. Upon oral administration, the compounds may be formulated into tablets or suitable forms. Examples of the parenteral administration of the novel compounds may include abdominal injection, hypothermic injection, intravenous injection and arterial injection for animals and intravenous or arterial injection and local injection for humans. The total administration amount and dose of the novel compounds of the present invention are dependent on administration routes and the patient or animal's conditions, such as age, body weight, sex, sensitivity, diet, administration time, co-administered medicines, severity, etc. Where the compounds of the present invention are used as antitumor agents, they can be administered at a wider range of doses than can adriamycin, and are preferably used at a dose of 5.0 to 25 mg/kg of body weight per day.

In addition, the compounds of the general formula I exhibit antibacterial activity against gram positive bacteria and thus, can be used

for treating the diseases caused by gram positive bacteria, at such a dose in the administration routes as described above.

Industrial Applicability

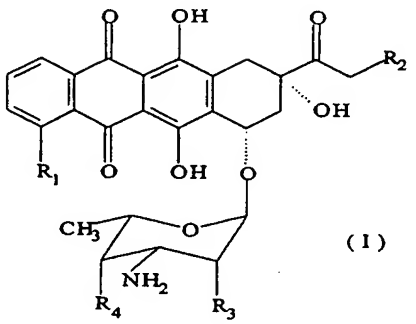
As described hereinbefore, the novel anthracycline compounds of the present invention have antitumor activity similar to that of conventional anthracycline drugs, but are extremely lower in cardiac toxicity than the conventional drugs. Thus, the novel anthracycline derivatives according to the present invention can be used as antitumor agents applicable for clinical practice. Also, because the anthracycline compounds represented by the general formula I exist as salt states, they show a high solubility in water and a high chemical stability in addition to being easy to chemically handle.

With low cardiac toxicity, the compounds of the present invention exhibit superb antitumor activity against cells and animal tumors, so they can be used as antitumor agents to treat malignant tumors, such as solid cancers and ascites cancers.

The present invention has been described in an illustrative manner, and it is to be understood the terminology used is intended to be in the nature of description rather than of limitation. Many modifications and variations of the present invention are possible in light of the above teachings. Therefore, it is to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

CLAIMS

1. Anthracycline glycoside derivatives, represented by the following structural formula I:



(I)

wherein R_1 and R_4, which may be the same or different, each is a hydrogen atom, methoxy or hydroxy;

R_2 is L-aspartate or pyruvate; and

R_3 is a hydrogen atom or fluorine atom.

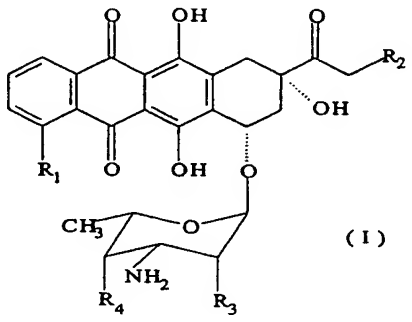
2. Anthracycline glycoside derivatives according to claim 1, wherein said anthracycline glycoside derivatives are in the form of non-toxic pharmaceutically acceptable acid-addition salts, combined with a pharmaceutically acceptable carrier or diluent.

3. An anticancer agent, comprising as an active ingredient an anthracycline glycoside derivative, represented by the formula I as claimed in claim 1, or acid addition salts thereof as claimed in claim 2.

4. A method of administering an antitumor agent as claimed in claim 3 comprising pharmaceutical composition in unit dosage form containing from 2 to 20 times the conventional dosage of adriamycin.

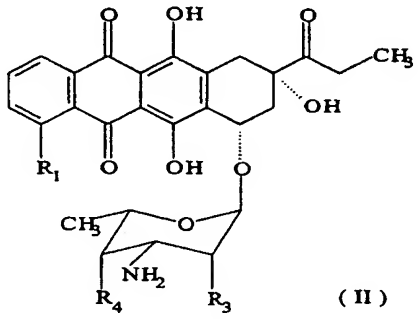
5. An antibiotic agent, comprising as an active ingredient an anthracycline glycoside derivative, represented by the formula I as claimed in claim 1 or acid addition salts thereof as claimed in claim 2 in an amount effective for inhibiting the growth of microorganisms.

6. A method for preparing an anthracycline derivative, represented by the following structural formula I:



(I)

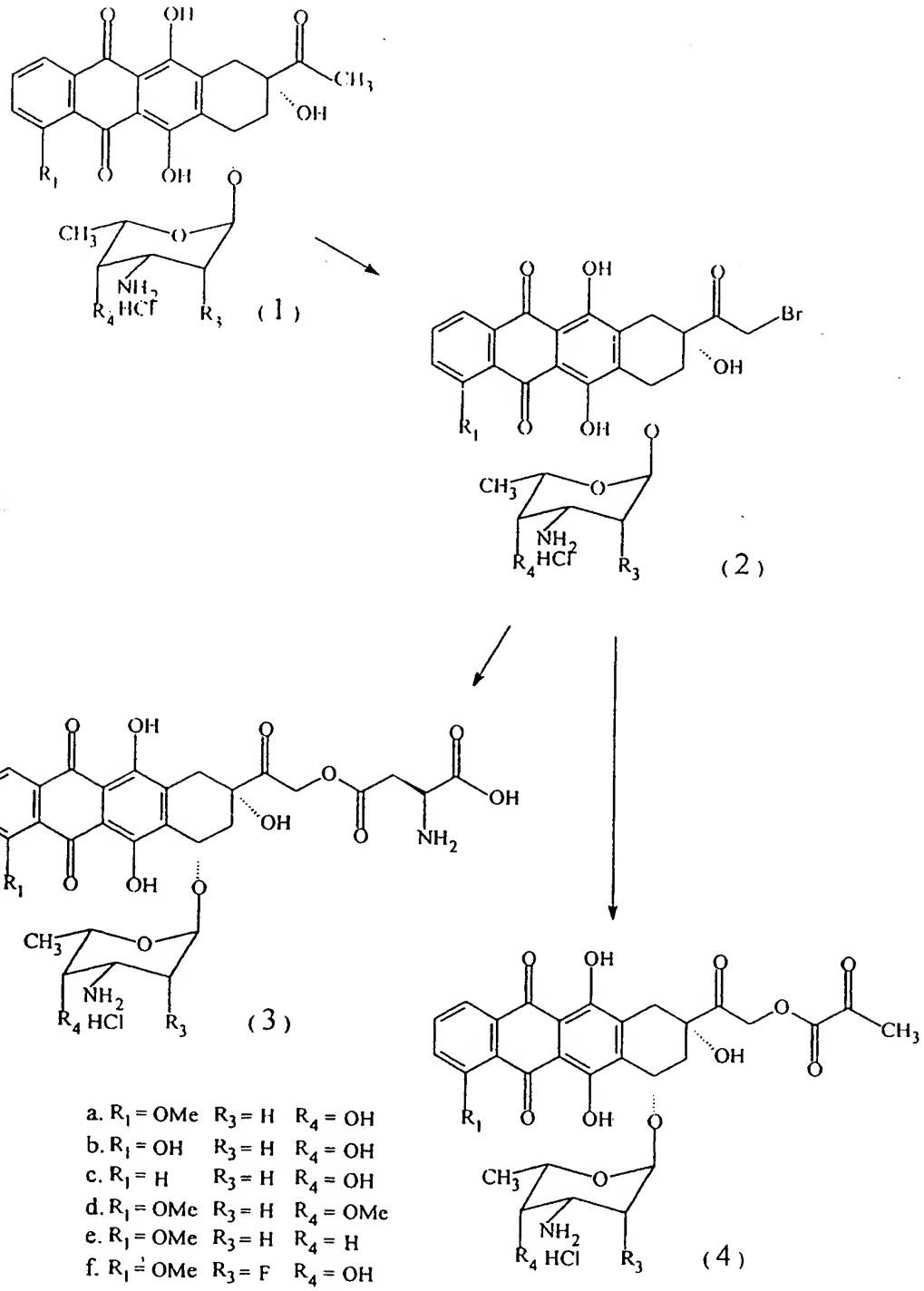
wherein R_1 and R_4, which may be the same or different, each is a hydrogen atom, methoxy or hydroxy; R_2 is L-aspartate or pyruvate; and R_3 is a hydrogen atom or fluorine atom, which comprises esterifying a compound, represented by the following formula II:



(II)

wherein R_1, R_3, and R_4 each are as defined above, with an L-aspartate or pyruvate.

FIG.1


(1)
(2)
(3)
(4)
a. R_1 = OMe R_3 = H R_4 = OH
b. R_1 = OH R_3 = H R_4 = OH
c. R_1 = H R_3 = H R_4 = OH
d. R_1 = OMe R_3 = H R_4 = OMe
e. R_1 = OMe R_3 = H R_4 = H
f. R_1 = OMe R_3 = F R_4 = OH

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 99/00220

A. CLASSIFICATION OF SUBJECT MATTER

IPC[6]: C 07 H 15/252; A 61 K 31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[6]: C 07 H; A 61 K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 646 177 A (KOCH et al.), 08 July 1997 (08.07.97), totality.	1-3,5

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
„A" document defining the general state of the art which is not considered to be of particular relevance
„E" earlier application or patent but published on or after the international filing date
„L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
„O" document referring to an oral disclosure, use, exhibition or other means
„P" document published prior to the international filing date but later than the priority date claimed

„T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
„X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
„Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
„&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 June 1999 (28.06.99)	01 September 1999 (01.09.99)

Name and mailing adress of the ISA/AT	Authorized officer
Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/200	Schnass Telephone No. 1/53424/217

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 99/00220

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 4 see remark:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 4 concerns a treatment of the human or animal body by therapy (Article 17(2)(a)(i) with Rule 39.1 (iv) PCT) this claim was searched, too.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims: it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR 99/00220

In Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
US A 5646177	08-07-1997	IT AO RM 940269 IT A 1272234	02-05-1994 16-06-1997

Form PCT/ISA/210 (patent family annex) (July 1998)